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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.:	10/809,946
Confirmation No.:	6414
Filing Date:	March 25, 2004
Examiner:	Deepak Rao
Group Art Unit:	1624
Applicants:	Guy Benchley et al.
For:	THIAZOLES USEFUL AS INHIBITORS OF PROTEIN KINASES

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF FRANCESCO G. SALITURO UNDER 37 C.F.R. § 1.132

I, FRANCESCO G. SALITURO, a citizen of the United States of America, residing at 25 Baker Dr., Marlborough, MA (US), hereby declare that:

1. I am one of the named inventors of the above-identified patent application.

2. I received a B.S. of Life Science at the University of Wisconsin in 1980. In 1984, I received a Ph.D. in Medicinal Chemistry from the University of Wisconsin. After receiving my Ph.D., I was a Post-Doctoral Research Associate at the University of Illinois from 1984 to 1986. From 1986 to 1993 I was a Senior Research Chemist and then a Senior Associate Scientist at Marion Merrell Dow Research Institute. In 1993, I joined Vertex Pharmaceuticals, Inc. (hereinafter "Vertex"). A copy of my curriculum vitae is attached as Exhibit B.

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3. From 2004 until present, I have been a Director of Medicinal Chemistry at Vertex. My work over the last 10 years has been devoted to the design and evaluation of protease and kinase inhibitors to treat human various human diseases, including rheumatoid arthritis, asthma, stroke, complications relating to organ transplantation, and cancer. I have co-authored 34 peer-reviewed papers and have been a co-inventor on 28 issued U.S. patents directed to small molecules useful in the treatment of human disease.

4. I am familiar with the August 10, 2006 and January 23, 2007 Office Actions in the above-identified application. I understand that, in the Examiner's view, claims 1, 7-19, 31-40, 43-44, and 46-47 of the present invention are rejected under 35 U.S.C. § 103(a) as being obvious and therefore unpatentable over Cochran et al., International Application Publication No. WO 02/096905, (hereafter "Cochran"). Specifically, the Examiner states that the unsubstituted 4-(thiazol-2-yl)pyrimidine compounds taught by Cochran are structural homologs of the compounds of the invention where the thiazol-2-yl group is substituted by a methyl group. Therefore, the Examiner asserts that it would have been obvious to one skilled in the art at the time of the invention to prepare the compounds of the present invention because such structurally homologous compounds would be expected to possess similar properties.

5. Mast cell degranulation assays are recognized as a reliable indicator of how effectively compounds inhibit the release of histamine from mast cells, and thus is a metric of their potency for treating an allergic reaction. I make this declaration to provide experimental results that relate to the concentration required to inhibit the degranulation of mast cells *in vitro* by 50% (hereinafter "IC_{50}") for certain compounds of the present invention. The thiazole compounds used for comparison were chosen such that the structures have identical substituents at the thiazole 2-position, but differ in the substituents at the thiazole 4- or 5-positions. The compounds taught by Cochran are unsubstituted at the 4- and 5-position, whereas the compounds of the invention have a methyl or hydroxymethyl substituent at the 4- or 5-position. Thus, the IC_{50} data provided

enables a head-to-head comparison of compounds taught by Cochran and closely-related compounds of the present invention. The IC_{50} data demonstrate that the compounds of the present invention have enhanced activity against the degranulation of mast cells as compared to compounds taught by Cochran. See paragraphs 7 to 9 below.

6. The assays used to determine the IC_{50} values provided herein were run according to the following protocol: RBL-2H3 cells were pretreated for one hour with a compound at different concentrations in a buffered solution or with a control (buffered solution without the compound). The cells were then co-stimulated with anti-dinitrophenyl (DNP) IgE and DNP-bovine serum albumin (BSA) for one hour. Beta hexosiminidase release was measured by substrate reaction with para-nitrophenyl-N-acetyl-beta-D-glucosaminide. Cell degranulation was measured spectrophotometrically for cells treated with the compound vs. those treated with the control. Spectrophotometric results obtained from unstimulated cells were used to provide background values and subtracted. IC_{50} values were generated using a 4-parameter curve fit in SoftMax Pro software package.

7. I have attached hereto Exhibit A, providing IC_{50} data for mast cells tested with selected compound species using the assay described above. These species include compound numbers I-109, I-144, I-146, I-82, I-83, and I-84 of the present invention (see Table 1 in the specification at pages 30-58) and Compounds A and B covered generically by formula I of Cochran (see the specification from page 7, line 8, to page 9, line 8).

8. Representative Compound A of Cochran (see Exhibit A) inhibited the degranulation of mast cells with an IC_{50} of 0.48 μmol. By comparison, compound no. I-109 of the present application showed mast cell inhibitory activity with an IC_{50} of 0.18 μmol. Furthermore, compounds I-144 and I-146 of the present invention showed mast cell inhibitory activities with an IC_{50}'s of 0.16 μmol and 0.12 μmol, respectively. These data indicate that replacement of a hydrogen atom on the thiazole ring of the compounds

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taught by Cochran with a methyl or hydroxymethyl group results in compounds of the invention that are two to three times more potent in the inhibition of mast cell degranulation.

9. Similarly, representative Compound B of Cochran (see Exhibit A) inhibited the degranulation of mast cells with an IC_{50} of 0.49 μmol, while compound no. I-82 of the present application showed mast cell inhibitory activity with an IC_{50} of 0.22 μmol. In addition, compounds I-83 and I-84 of the present invention showed mast cell inhibitory activities with an IC_{50}'s of 0.08 μmol and 0.16 μmol, respectively. These data indicate that replacement of a hydrogen atom on the thiazole ring of the compounds taught by Cochran with a methyl or hydroxymethyl group results in compounds of the invention that are at least two to three times more potent in the inhibition of mast cell degranulation.

10. I declare further that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that such willful false statements may jeopardize the validity of this application and any patent issuing thereon.

Francesco G. Salituro

Signed this 6th of March, 2007
At Cambridge, MA, USA.

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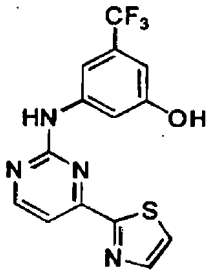
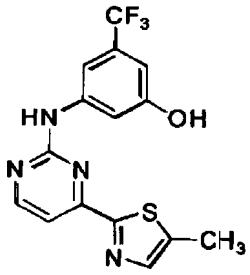
Exhibit A

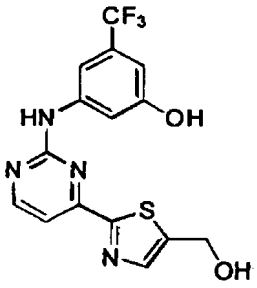
IC_{50} Values for the Inhibition of Mast Cell Degranulation



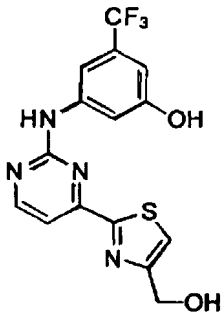
(Compound A)
Cell Degranulation
IC_{50} = 0.41 μM



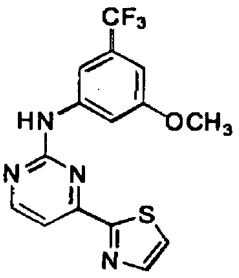
(I-109)
Cell Degranulation
IC_{50} = 0.18 μM



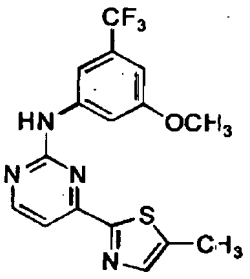
(I-144)
Cell Degranulation
IC_{50} = 0.16 μM



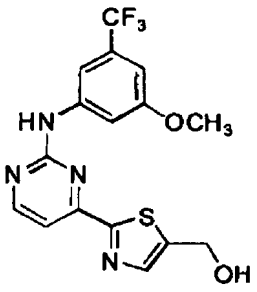
(I-146)
Cell Degranulation
IC_{50} = 0.12 μM



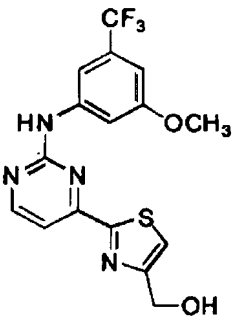
(Compound B)
Cell Degranulation
IC_{50} = 0.49 μM



(I-82)
Cell Degranulation
IC_{50} = 0.22 μM



(I-83)
Cell Degranulation
IC_{50} = 0.08 μM



(I-84)
Cell Degranulation
IC_{50} = 0.16 μM

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Exhibit B

Curriculum Vitae of Francesco G. Salituro

EDUCATION AND EXPERIENCE:

Present

Director, Medicinal Chemistry
Vertex Pharmaceuticals

- Position re-titled in Q4/04 to Director of Medicinal Chemistry, overseeing approximately 1/3 of Vertex Chemistry Department
- Manage drug discovery efforts in kinase research, 13 Vertex chemists (7 Ph.D. and 6 MS) and 4 external chemists
- Identifying and managing outsource collaborations
- Program strategic planning, prioritization and resource allocation
- Recruiting, hiring, staffing
- Cost center budget responsibilities

2001-2004

Principal Investigator
Vertex Pharmaceuticals

- Promoted to Principal Investigator in early 2001
- Continued Project Leader for SAPK kinase program and subsequently, two additional kinase programs, towards developing pharmaceutical agents for stroke, transplantation, asthma and cancer
- Coordinated a multi-disciplinary Matrix team (15-20 scientists)
- Lead programs from early phase discovery through animal pharmacology and pre-clinical toxicology
- Involved with early business development activities
- Co-responsibility as Chemistry Team Leader overseeing activities for 10 chemists
- Recruiting and hiring responsibilities

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1997-2001

Senior Staff Investigator
Vertex Pharmaceuticals

- Chemistry team leader in p38 Map Kinase project leading to the discovery of a clinical candidate for Rheumatoid Arthritis, VX-745, in addition to a second generation series class which resulted in a second clinical candidate VX-702. Managed internal and external chemistry team of 10+ chemists
- Promoted to Project Leader for SAPK Kinase program in 1998 (matrix team of 15-20)
- Involved with early business development activities for developing potential partnerships for SAPK
- Recruiting and hiring responsibilities

1993-1997

Staff Scientist
Vertex Pharmaceuticals

- Hired as Staff Scientist in September 1993
- Chemistry Group Leader for HIV Protease 2nd generation project (through September1996) and for p38 Map Kinase project (1996-1998). Supervised chemistry groups consisting of 7 internal members, (Ph.D. and non-Ph.D.) and coordinated chemistry research collaborations with corporate partners on both HIV and P38 programs (total chemistry group members ranged from 7-12).
- Involved with early business development activities for developing corporate partnerships in both HIV and p38.
- Recruiting and hiring responsibilities.

1990 – 1993

Sr. Associate Scientist
Marion Merrell Dow Research Institute
Primary responsibilities from 1986-1993: Bench chemist supervising one Masters level chemist.

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	Research focus on the design and synthesis of NMDA Glycine Site Antagonists.
1992	**Department Safety Representative**
1988 - 1991	**Assistant Safety Representative** Department of Discovery Chemistry
1986 - 1989	**Sr. Research Chemist I** Merrell Dow Research Institute
1984 - 1986	**UNIVERSITY OF ILLINOIS, Champaign-Urbana,** Post-Doctoral Research Associate with Professor John A. Katzenellenbogen. Synthesis of [^{125}I]iodota-moxifen aziridine, a non-steroidal, anti-estrogenic affinity label and radiolabel for the estrogen receptor. Synthesis of haloenol lactone inhibitors of elastase.
1980 - 1984	**UNIVERSITY OF WISCONSIN-Madison; Madison, Wisconsin.** **Ph.D. (Medicinal Chemistry); October, 1984.** Dissertation research, under the direction of Professor Daniel H. Rich, involved synthetic and kinetic studies of pepstatin analogs as potent, selective aspartyl protease inhibitors.
1982 - 1983	Served as substitute lecturer for an undergraduate Medicinal Chemistry course at the University of Wisconsin-Madison.
1976 - 1980	**UNIVERSITY OF WISCONSIN-Parkside; Kenosha, Wisconsin.** **B.S. (Life Science),** Magna Cum Laude, 1980. Undergraduate research (1978-1980) in Bioorganic Chemistry under Professor Bruce R. Branchini. participated in a project aimed at the synthesis, development and kinetic analysis of chemiluminescent substrates for serine proteases.

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PUBLICATIONS:

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2. "Sensitive Enzyme Assays Based on the Production of Chemiluminescent Leaving Groups," B.R. Branchini, J.D. Hermes, **F.G. Salituro**, N.J. Post, and G. Claeson, Anal. Biochem., 111, 87 (1981).

3. "Conformational Flexibility in the Active Sites of Aspartyl Proteinases Revealed by a Pepstatin Fragment Binding to Penicillopepsin," M.N.G. James, A. Sielecki, **F.G. Salituro**, D.H. Rich, and T. Hofmann, Proc. Natl. Acad. Sci. USA, 79, 6137 (1982).

4. "Synthesis of Analogs of Pepstatin. Effect of Structure in Subsites $P_{1'}$, P_2 and P_2 on Inhibition of Porcine Pepsin," D.H. Rich, **F.G. Salituro**, J. Med. Chem., 26, 904 (1983).

5. "Design of Protease Inhibitors." D.H. Rich, **F.G. Salituro**, and M.W. Holladay in "Peptides: Structure and Function. Proc. Eighth American Peptide Symposium," V. Hruby and D.H. Rich (eds.), Pierce Chemical Co., Rockford, IL, p. 511.

6. "Design and Discovery of Aspartyl Proteasee Inhibitors. Mechanism and Clinical Implicaitons," D.H. Rich, **F.G. Salituro**, and M.W. Holladay, in "Drug Design Based on Peptide and Nucleic Acid Conformational Structure," J. Vida (ed.), Academic Press, N.Y., N.Y., pp. 213-237.

7. "Inhibition of Aspartyl Proteases by Me^3Sta Derivatives of Pepstatin. Evidence for a Collected-Substrate Mechanism of Enzyme Inhibition," D.H. Rich, M.S. Bernatowicz, N.S. Agarwal, M. Kawai, **F.G. Salituro**, and P.D. Schmidt, Biochemistry, 24, 3165 (1985).

8. "Identification of Oxygen Nucleophiles in Tetrahedral Intermediates: ^2H and ^{18}O Induced Isotope Shifts in ^{13}C NMR Spectra of Pepsin-Bound Peptide Ketone Pseudosubstrates," P.G. Schmidt, M.W.

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9. "Pepsin-Catalyzed Addition of Water to a Ketomethylene Peptide Isostere. Observation of the Tetrahedral Species by ^{13}C NMR," M.W. Holladay, **F.G. Salituro**, P.G. Schmidt, and D.H. Rich, Biochemical Society Transactions, 13, 1046, 1985.

10. "Inhibition of Aspartic Proteinases by Lysine and Ornithine Side chain Analogs of Statine," **F.G. Salituro**, N.A. Agarwal, T. Hofmann, D.H. Rich, J. Med. Chem., 30, 286 (1987).

11. "Synthetic and Porcine Pepsin Inhibition Studies of Pepstatin Aanlogs Containing Ketomethylene and Hydroxyethylene Dipeptide isosteres," M.W. Holladay, **F.G. Salituro**, D.H. Rich, J. Med. Chem., 30, 374 (1987).

12. "[^{125}I]Iododesythyl Tamoxifen Aziridine: Synthesis and Covalent Labeling of the Estrogen Receptor with an Iodine-Labeled Affinity Label," **F.G. Salituro**, K.E. Carlson, J.F. Elliston, B.S. Katzenellenbogen, and J.A. Katzenellenbogen, Steroids, 48 (5-6), 287 (1986).

13. "Facile Synthesis of L-kynurenine," **F.G. Salituro**, I.A. McDonald, J. Org. Chem., 53, 6138 (1988).

14. "3-(2-Carboxyindol-3-yl) propionic Acid Derivatives: Antagonists of the Strychnine-Insensitive Glycine Receptor Associated with the NMDA Receptor Complex." **F.G. Salituro**, B.L. Harrison, B.M. Baron, P.L. Nyce, K.T. Stewart, I.A. McDonald, J. Med. Chem. 33, 2944-2946 (1990).

15. "Activity of 5,7-Dichlorokynurenic Acid, a Potent Antagonist at the NMDA Receptor-Associated Glycine Binding Site." B.L. Baron, B.L. Harrison, F.P. Miller, I.A. McDonald, **F.G. Salituro**, C.J. Schmidt, S.M. Sorensen, H.S. White, M.G. Palfreyman, Mol. Pharmacol., 38, 554-561 (1990).

16. "Design, Synthesis and Molecular Modeling of 3-Acylamino-2-Carboxyindole NMDA Receptor Glycine-Site Antagonists." **F.G. Salituro**, R.C. Tomlinson, B.M. Baron, D.A. Demeter, H.J.R.

Weintraub and I.A. McDonald, BioMed. Chem. Lett., 1, 455-460 (1991).

17. "3-(2-Carboxyindol-3-yl)Propionic Acid-Based Antagonists of the NMDA Receptor Associated Glycine Site." **F.G. Salituro**, B.L. Harrison, B.M. Baron, P.L. Nyce, K.T. Stewart, J.H. Kehne, H.S. White and I.A. McDonald, J. Med. Chem., 35, 1792-1799 (1992).

18. "Potent Indole- and Quinoline-Containing N-Methyl-D-Aspartate Antagonists Acting at the Strychnine-Insensitive Glycine Binding Site." B.M. Baron, B.L. Harrison, I.A. McDonald, B.S. Meldrum, M.G. Palfreyman, **F.G. Salituro**, S.W. Siegel, A.L. Slone, J.P. Turner and H.S. White, J. Pharmacol. Exp. Ther., 262, 947-956 (1992).

19. "The Design of NMDA Receptor Glycine-Site Antagonists," **F.G. Salituro**, I.A. McDonald, B.L. Harrison, Drug News and Perspectives, 6 215-223 (1993).

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21. "Design and Pharmacological Evaluation of Highly Selective Glycine Anagonists," B.B. Baron, J.H. Kehne, S.M. Sorensen, B.L. Harrison, **F.G. Salituro**, H.S. White,in Direct and Allosteric Control of Glutamete Receptors; CRC Press, 1994 p 105-117.

22. "Multisubstrate Inhibition of 4-Hydroxybenzoate 3-Monooxygenase," **F.G. Salituro**, D.D. Demeter, H.J.R. Weintraub, B.J. Lippert, R.J. Resvick, I.A. McDonald, J. Med. Chem., 37, 4076-4078 (1994).

23. "MDL 100,458 and MDL 102,288: Two potent and Selective Glycine Antgonists With Different Functional Profiles," J.H. Kehne, B.B. Baron, B.L. Harrison, T.C. McCluskey, M.G. Palfreyman, M. Poirot, **F.G. Salituro**, B.W. Siegel, A.L. Slone, P. van Giersbergen, H.S. White, Eur. J. Pharmacol., 284, 109-118 (1995)

24. "Pharmacological Characterization of MDL 105,519, an NMDA receptor glycine site antagonist" B.M.Baron, B.L. Harrison, J.H. Kehne, C.J. Schmidt, P. van Giersbergen, H.S. White, B.W. Siegel, Y.

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27. "Pharmacological characterization of MDL 105,519, an NMDA receptor glycine site antagonist."Baron BM, Harrison BL, Kehne JH, Schmidt CJ, van Giersbergen PL, White HS, Siegel BW, Senyah Y, McCloskey TC, Fadayel GM, Taylor VL, Murawsky MK, Nyce P, **Salituro FG**. Eur J Pharmacol. 1997 Apr 4;323(2-3):181-92.

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29. "Design and synthesis of novel conformationally restricted HIV protease inhibitors." **Salituro FG**, Baker CT, Court JJ, Deininger DD, Kim EE, Li B, Novak PM, Rao BG, Pazhanisamy S, Porter MD, Schairer WC, Tung RD., Bioorg Med Chem Lett. 1998 Dec 15;8(24):3637-42.

30. "Inhibitors of p38 MAP kinase: therapeutic intervention in cytokine-mediated diseases."**Salituro FG**, Germann UA, Wilson KP, Bemis GW, Fox T, Su MS. Curr Med Chem. 1999 Sep;6(9):807-23.

31. "Novel inhibitors of HIV protease: design, synthesis and biological evaluation of picomolar inhibitors containing cyclic P1/P2 scaffolds." Spaltenstein A, Almond MR, Bock WJ, Cleary DG, Furfine ES,

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Hazen RJ, Kazmierski WM, **Salituro FG**, Tung RD, Wright LL. Bioorg Med Chem Lett. 2000 Jun 5;10(11):1159-62.

32. "Structure, synthesis, and biological activity of VX-745: A novel, orally bioavailable and selective p38 MAP kinase inhibitor" **Francesco G. Salituro,** Guy W. Bemis, Ursula A.Germann, John P. Duffy,Vincent P. Galullo, Edmund M. Harrington, S. Pazhanisamy, Pamela J. Ford, Karyn L. Cepek, Yow-Ming C. Wang, Steven F. Bellon, George Ku, Keith P.Wilson, Michael S.-S Su. Actualité de Chimie therapeutique, 2002, 28th series, pp173-189.

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U.S. PATENTS:

1. 7,115,637 Inhibitors of p38

2. 6,800,626 Inhibitors of p38

3. 6,693,108 Inhibitors of c-JUN N terminal kinases (JNK) and other protein kinases

4. 6,689,778 Inhibitors of Src and Lck protein kinases

5. 6,635,644 Inhibitors of p38

6. 6,632,945 Inhibitors of p38

7. 6,608,060 Inhibitors of p38

8. 6,528,508 Inhibitors of p38

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9. 6,509,363 Heterocyclic inhibitors of p38

10. 6,147,080 Inhibitors of p38

11. 6,127,372 Sulfonamide inhibitors of aspartyl protease

12. 6,093,742 Inhibitors of p38

13. 5,945,418 Inhibitors of p38

14. 5,945,413 Aspartyl protease inhibitors

15. 5,883,252 Aspartyl protease inhibitors

16. 5,877,202 Indole derivatives useful to treat estrogen-related neoplasms and disorders

17. 5,703,107 3-aminoindolyl derivatives

18. 5,675,018 3-amidoindolyl derivatives

19. 5,547,991 NMDA antagonism method

20. 5,519,048 3-(indol-3-yl)-propenoic acid derivatives and pharmaceutical compositions thereof

21. 5,491,153 3-amidoindolyl derivative

22. 5,484,814 NMDA antagonists

23. 5,470,870 NMDA antagonists

24. 5,360,814 NMDA antagonists

25. 5,189,054 3-amidoindolyl derivatives and pharmaceutical compositions thereof

26. 5,106,847 Excitatory amino acid antagonists, compositions and use

27. 5,051,442 3-indolyl thioacetate derivatives and NMDA receptor antagonistic use thereof

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28. 4,960,786 Excitatory amino acid antagonists